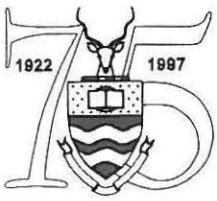
The systematic value of the surface micromorphology and anatomy of cypselae of some members of the Senecioneae, Liabeae and Vernonieae (Asteraceae)



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Received 23 April 1997; revised 26 August 1997

Twenty one characters of the surface micromorphology and 12 characters of the anatomy of cypselae of species in the tribes Liabeae (*Liabum discolor*, *L. ovatum*), Senecioneae (subtribe Senecioninae: *Senecio inaequidens*, *S. madagascariensis*, *S. vulgaris*, *Brachyglottis repanda*, subtribe Blennospermatinae: *Abrotanella emarginata*, *A. scapigera*, *Blennosperma nanum*) and Vernoneae (*Vernonia oligocephala*, *V. pauciflora*, *V. poskeana*) were investigated using light microscopy (LM) and scanning electron microscopy (SEM). The data were analysed phenetically with the assistance of cluster (SAHN) and principal component analyses. Both sources of data were biologically very informative. Taxonomically, the SEM data was most useful in recognising discontinuities between the taxa followed by the combined SEM and LM data sets. The placement of the Blennospermatinae in the Senecioneae is confirmed as being systematically discordant.

Keywords: Cypselae, micromorphology, anatomy, Asteraceae.

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Introduction

The Compositae or Asteraceae is one of the largest and most familiar families of flowering plants and has been researched for centuries (Heywood *et al.* 1977).

A diverse range of characters has been used to delimit the family. Micromorphological characters of the florets have played an important role in the systematics of the Asteraceae since the investigations of Cassini (Wagenitz 1976). The basic plan of the wood anatomy of the family has also provided useful characters, although little intertribal variation has been found (Carlquist 1966). Embryology (e.g. Pandoy & Singh 1980), phytochemistry (e.g. Robins 1977), cytology, palynology (Vincent & Getliffe Norris 1989), micromorphology (e.g. Nordenstam 1978; Vincent & Getliffe 1988, 1992; Wagenitz 1976; Wetter 1983) and molecular studies (e.g. Baldwin 1992, 1993) have all provided considerable data for systematic studies of various taxa in the Asteraceae.

However, the anatomy of the fruits (cypselae), sometimes erroneously referred to as achenes (Pope 1983), has not been investigated widely in the family. According to Wagenitz (1976) the study of the fruit anatomy (termed carpology) is a promising field for elucidative studies on the systematics of the Asteraceae. The systematic importance of cypsela anatomy of the Asteraceae has been demonstrated in the Anthemideae by Bruhl and Quinn (1990) and Källersjö (1985). Roth (1977) has provided considerable evidence on the use of cypsela anatomy data in systematics. Bremer (1987) stated that cypselae provide a wealth of character information at the lower taxonomic levels and that variation in morphology is so great that it is not very useful at the tribal level. Note that the use of the term cypsela here is as defined by Jackson (1949).

The cypsela is a special form of dry indehiscent fruit in which the seed coat (testa) and fruit wall (pericarp) are tightly attached to one another and is characteristic of the family Asteraceae (Roth 1977).

The cypselae of the Asteraceae are usually elongate in shape, and are often compressed and show a characteristic sculpturing on their surface. Appendages are usually present and may be in the form of enlarged ribs, wings or hooks, specialized epidermal outgrowths such as protuberances, special trichomes, or mucilaginous cells (Roth 1977).

Nevertheless, characters dealing with surface micromorphology and anatomy of cypselae of the Asteraceae have not been investigated very much. The purpose of this study of Asteraceae carpology was primarily to evaluate a wide range of micromorphological and anatomical characters of the cypselae of certain members of the Senecioneae, Liabeae and Vernonicae (Appendix 1). Furthermore, the data was used to elucidate the systematic affinities of the taxa investigated as the systematic affinity of these three tribes has been the subject of much argument (Bremer 1994).

Materials and Methods

The choice of the three tribes included in this study is based primarily on recent argument concerning the systematic affinities of these three tribes (Bremer 1994). The taxa investigated are representatives of the tribes Senecioneae (subtribes Senecioninae & Blennospermatinae), Liabeae and Vernoneae (Appendix 1).

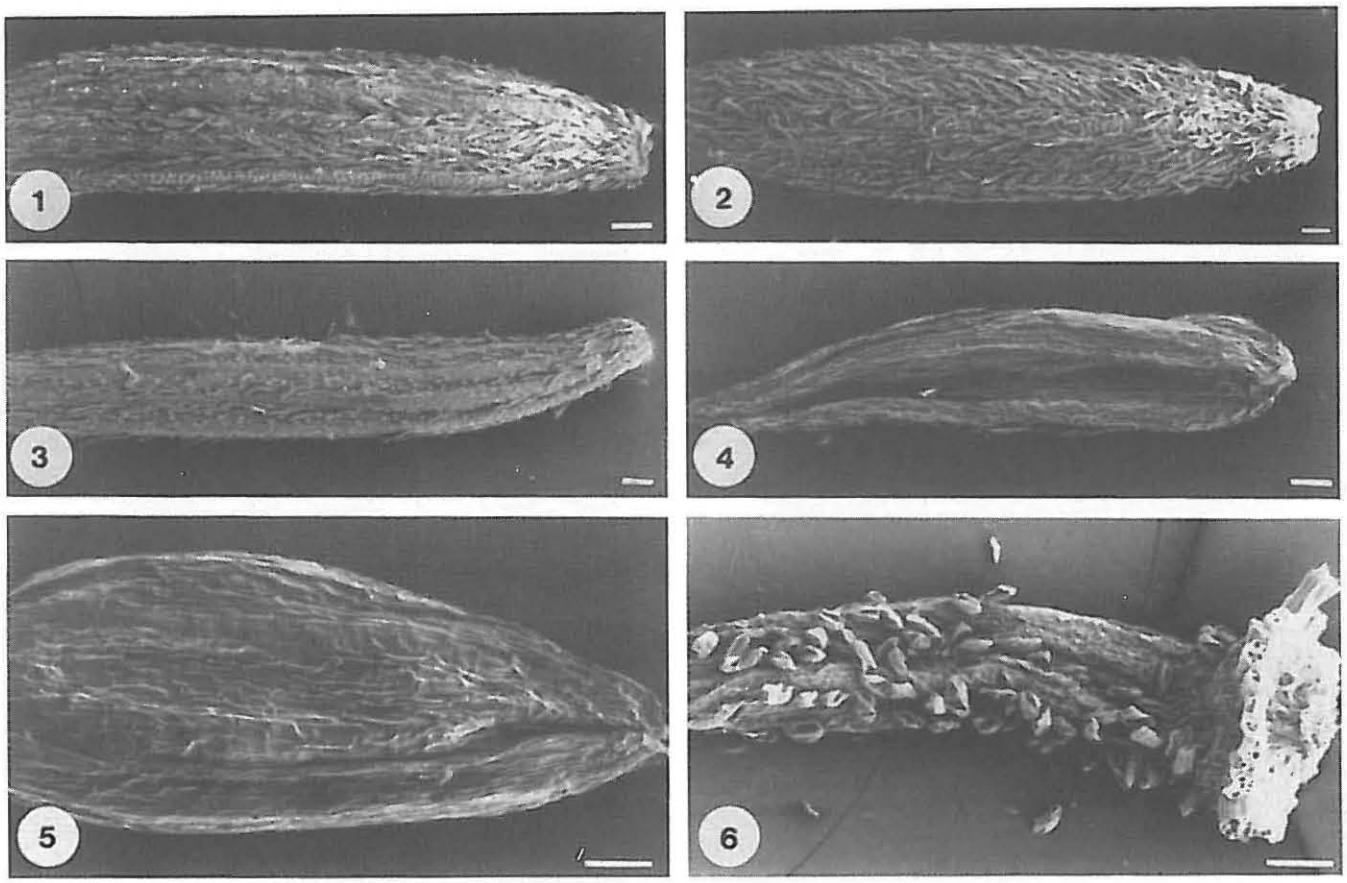
As little or no information is available as to which species is morphologically the most representative of each of the tribes and subtribes investigated (Appendix 1), it was decided to obtain cypselae from the type species of the type genus wherever possible, notwithstanding the fact that the type species is not necessarily the most representative taxon of the genus. Cypselae of putatively closely allied taxa to the various type species were also sampled. The herbarium material investigated was obtained from PRE and K (Appendix 1). Intraspecific variation was noted by sampling cypselae from two or three herbarium specimens of each of the species studied.

Scanning Electron Microscopy (SEM)

Dehydrated cypselae from herbarium specimens were secured directly on to aluminium viewing stubs using double-sided tape. The stubs were coated with gold/palladium using a Polaron sputter coater and viewed with a Jeol JSM 840 scanning electron microscope. Data were recorded from the photographs that were taken. Differences in magnification were taken into account when recording the data.

Light Microscopy (LM)

Several cypselae from each specimen were rehydrated in warm water (40°C) on a hot tray for three days and then cut in half.



Figures 1–6 Scanning electron micrographs of cypselae: **1**. *S. madagascariensis;* **2**. *S. inaequidens;* **3**. *S. vulgaris*; **4**. *A. scapigera;* **5**. *A.emarginata*; **6**. *B. repanda*. Scale bars: 0.1 mm.

transversely, to facilitate penetration of the fixatives. Fixation was done in formalin aceto-alcohol (FAA) overnight. The samples were then subjected to a graded alcohol (ethanol) dehydration series (70%, 80%, 90% & 95%) for 30 min each and then twice in absolute alcohol for 30 min each time. Initial embedding was in Spurr's resin, but it was found that the resin failed to infiltrate the tissues sufficiently for sectioning and viewing. Subsequent embedding was in LR White resin. Dehydrated cypselae were removed from the absolute alcohol and infiltrated in LR White resin:absolute alcohol (50:50) for 20 min and then in resin:absolute alcohol (67:33) for a further 20 min. Finally the cypselae were embedded in pure resin overnight after being placed in a vacuum oven at 100 mbar for 4–5 hours.

Transverse sections (1.0–2.5 µm thick) were cut using a Reichert 2030 microtome. In most cases good quality sections were obtained (see Appendix 1 for list of specimens that yielded good quality sections for LM). Sections were stained in Safranin and Fast Green (Johansen 1940). All observations were made using a Zeiss MC63 photo microscope.

Choice of characters investigated

Twenty one characters (Appendix 2 and 4) were recorded for each specimen (Appendix 1) using the SEM photographs. Twelve characters (Appendix 3 and 5) were recorded using the LM photographs.

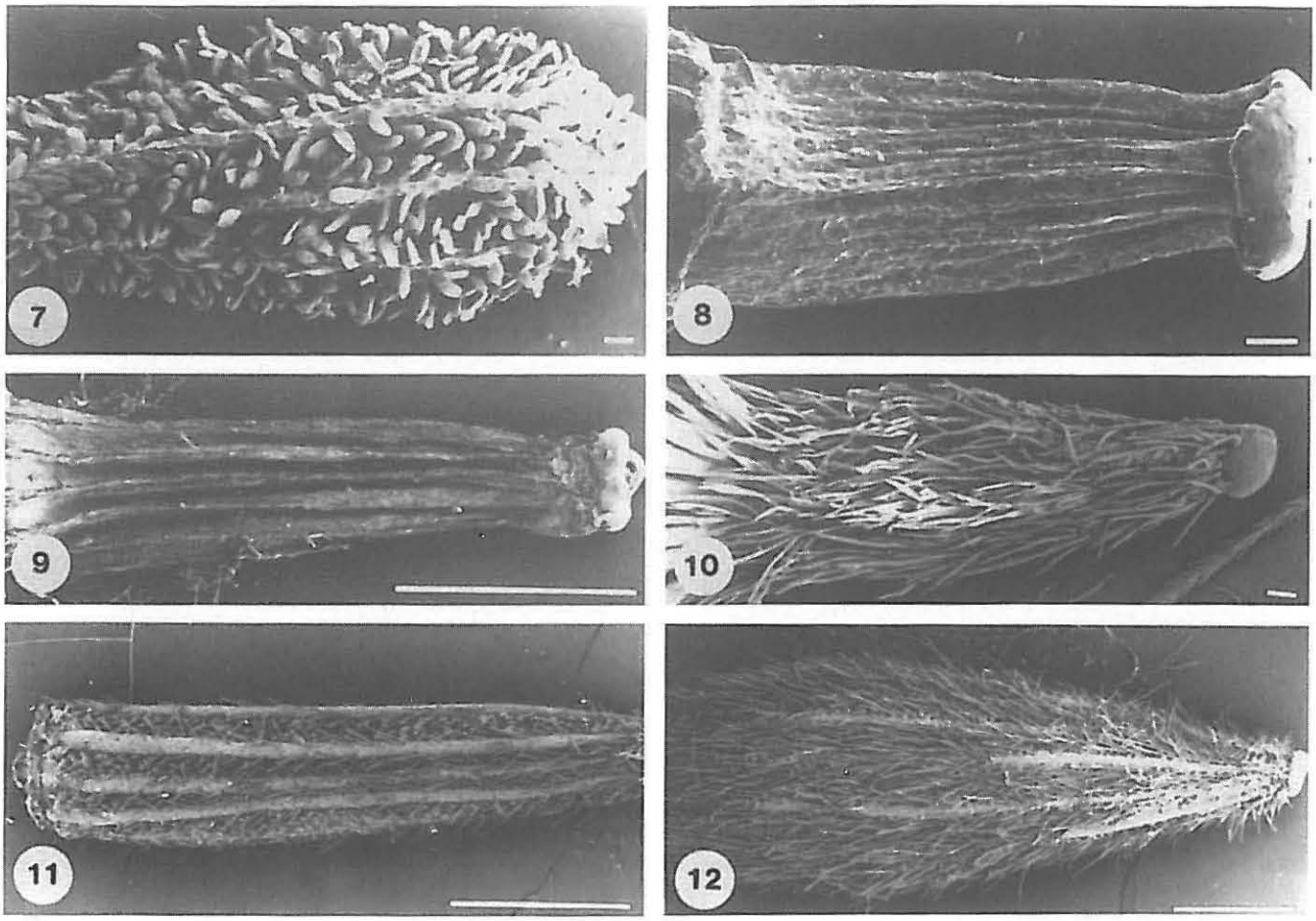
Analysis of data

Three t × n (taxon × character) data matrices were developed (SEM data, LM data and combined SEM & LM data - the combined data matrix is not provided here). Each data matrix was analysed using the numerical phenetics programme NTSYS-pc (Rohlf 1988). The data were standardized (Sneath & Sokal 1973) using the STAND subroutine, resulting in a mean of zero and a standard deviation of unity for each character. Standardization was performed since in most instances nominal-level (non-parametric) coding was used for the characters. Standardization enabled each character to contribute toward the overall resemblance inversely in proportion to its variability amongst the entire set of OTU's. Distance and correlation matrices were computed for each matrix using the SIMINT subroutine, to determine the similarity of the taxa in each matrix in terms of distance and correlation. This gives an indication of the inter-taxon phenetic relationship or affinity (Dunn & Everitt 1982). The SAHN subroutine was used to perform various clustering patterns (e.g. UPGMA, WPGMA). The CONSENSUS subroutine was used to produce a consensus tree when several, equally parsimonius trees were generated. The COPH subroutine was used to test the goodness of fit of the clustering matrices to the original distance or correlation matrices. The cophenetic correlation coefficient (r) was computed for each data set. Principle Components Analysis (PCA) (3 components) was performed to produce a visual representation of the putative phenetic relationships amongst the taxa (OTU's) as well as determining which characters are most important in defining these phenetic relationships between the taxa in low-dimensional space.

Results and Observations

Characters and Character States (Appendix 2) of the cypsela micromorphology recorded via the SEM study (character state abbreviated to 'c/state').

Shape of cypselae (SEM Char. 1): The variation in the shape of the cypselae of the taxa investigated was represented by 5 character states. The specimens of *S. madagascariensis* (Figure 1) and *S. vulgaris* (Figure 3) had narrowly elliptic cypselae (c/state 1). Those of *S. inaequidens* (Figure 2) were elliptic (c/state 2) - having a greater width than those described by c/state 1. The cypselae of *A. scapigera* (Figure 4), *B. repanda* (Figure 6), *L. discolor* (Figure 8), *L. ovatum* (Figure 9), *V. oligocephala*



Figures 7–12 Scanning electron micrographs of cypselae: **7**. *B. nanum*; **8**. *L. discolor*; **9**. *L. ovatum*; **10**. *V. oligocephala*; **11**. *V. poskeana*; **12**. *V. pauciflora*. Scale bars: **7**, **8** and **10** = 0.1 mm; **9**, **11** and **12** = 1.0 mm.

(Figure 10) and *V. poskeana* (Figure 11), were narrowly obovate (c/state 3). The cypselae of *A. emarginata* (Figure 5) were obovate (c/state 4), while those of *B. nanum* (Figure 7) and *V. pauciflora* (Figure 12) were ovate (c/state 5).

Length:Width ratio of cypselae (SEM Char. 2): Three size classes (character states) were defined on the basis of a frequency distribution of the length:width ratios of all the cypselae, such that each size class corresponded to a peak in the frequency distribution plot. Different specimens of the same species possessed cypselae with different length:width ratios (see Appendix 4).

Presence or absence of ribbing on the cypselae (SEM Char. 3): All of the species possessed ribs (c/state 0) except for *A. scapigera* (Figure 4) and *A. emarginata* (Figure 5), which, from the micrographs, appeared to be ribless.

Nature of ribbing (SEM Char. 4): In most of the taxa with ribbed cypselae, the ribs were of equal size (c/state 0). The cypselae of *L. ovatum* had alternating small and large ribs (c/state 1). This was confirmed by the light microscope observations (Figure 37).

Presence or absence of vestiture (SEM Char. 5): The cypselae of *A. scapigera* (Figure 16), *A. emarginata* (Figure 17), *L. discolor* (Figure 20) and *L. ovatum* (Figure 21) were glabrous (c/state 1). The cypselae of the remaining taxa all possessed vestiture of some sort (c/state 0). This ranged from the presence of trichomes of various types (SEM Char. 11) to the presence of verrucae (SEM Char. 7), papillae (SEM Char. 13) and large bulbous mucilaginous cells (SEM Char. 15).

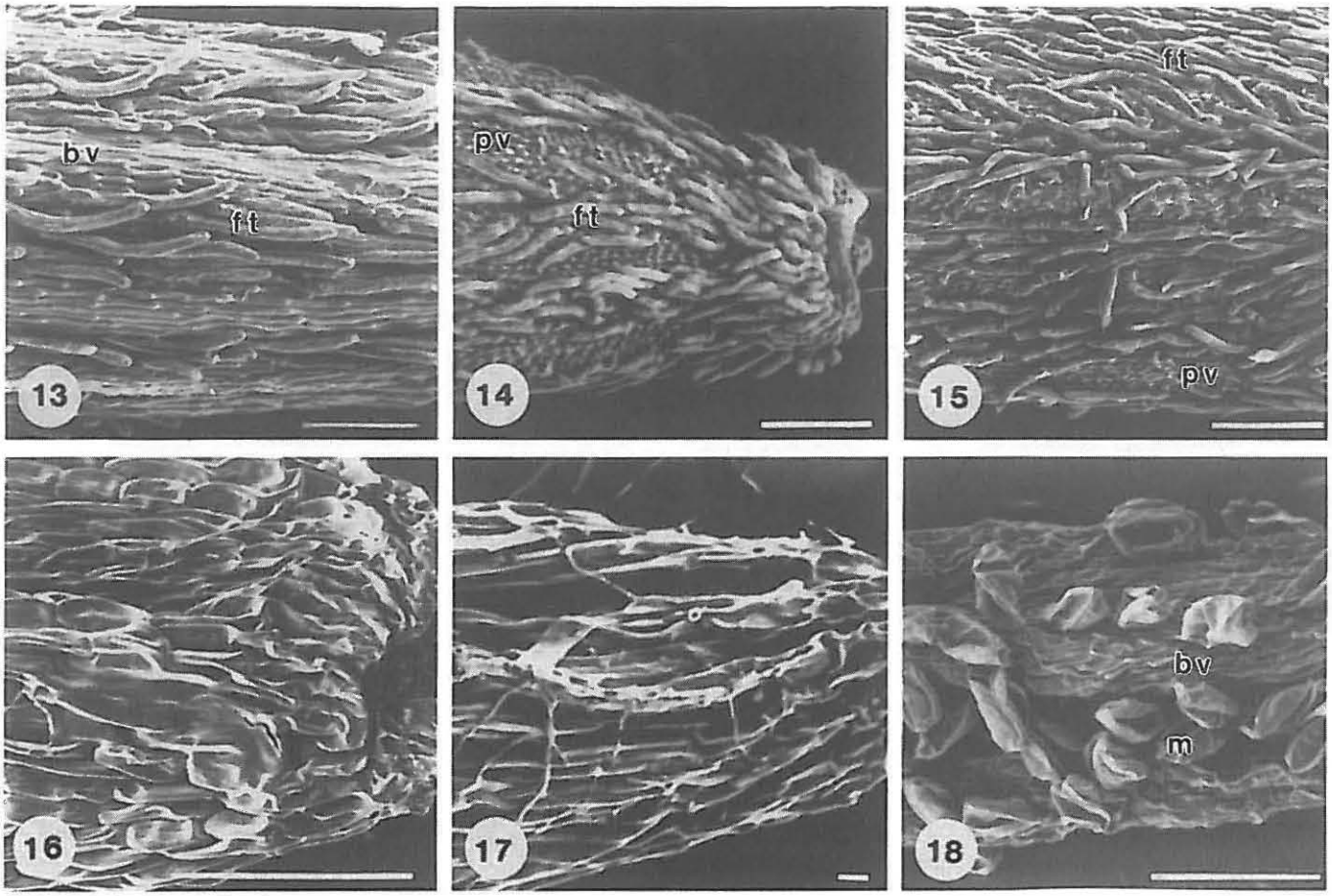
Number of different types of cells/trichomes forming the vestiture (SEM Char. 6): Only *B. nanum* possessed cypselae with only one type of trichome (c/state 1). The cypselae of the remaining taxa possessed two types of cells/trichomes (c/state 0).

Presence or absence of verrucae (SEM Char. 7): The cypselae of *S. vulgaris* (Figure 13), *S. madagacariensis* (Figure 14), *S. inaequidens* (Figure 15) and *B. repanda* (Figure 18) possessed short verrucae which were quite closely adpressed to the fruit surface (c/state 0). The remaining vestitured cypselae did not possess any verrucae (c/state 1).

Nature of the verrucae (SEM Char. 8): The verrucae on the cypselae of *S. vulgaris* (Figure 13) and *B. repanda* (Figure 18) were blunt-ended (c/state 0). Those of *S. madagascariensis* (Figure 14) and *S. inaequidens* (Figure 15) were pointed at their distal ends (c/state 1).

Location of the verrucae (SEM Char. 9): The verrucae on the cypselae of *B. repanda* (Figure 18) covered the entire fruit surface (c/state 1), while those of *S. vulgaris*, *S. madagascariensis* and *S. inaequidens* (Figures 13–15) were located only on the ridges of the cypselae (c/state 0).

Presence or absence of duplex hairs (SEM Char. 10): Twin hairs (or duplex trichomes) are very characteristic of the fruits of many of the Compositae (Roth 1977). Twin hairs develop from a single epidermal cell which divides longitudinally producing two cells which later divide to form two basal and two apical cells. It is the two apical cells which make up the majority of each twin hair. The cavities of the cells comprising the twin hairs may contain slime or mucilage. The twin hair cells may be united along



Figures 13–18 Scanning electron micrographs of cypselae showing external micromorphology: **13.** *S. vulgaris* - short, blunt-ended verrucae (**bv**) on ridges, and finger-like trichomes (**ft**) in furrows; **14.** *S. madagascariensis* and **15.** *S. inaequidens* - both with short, pointed verrucae (**pv**) on ridges and finger-like trichomes (**ft**) in furrows; **16.** *A. scapigera* and **17.** *A. emarginata*- cypselae glabrous; **18.** *B. repanda* - short, blunt-ended verrucae (**bv**) cover cypsela surface and large, bulbous mucilaginous cells (**m**) (collapsed here) scattered over surface. Scale bars: **13–16** and **18** = 0.1 mm; **17** = 0.01 mm.

their whole length, or they may be separated at their apex to form a forked trichome. Forked trichomes are characteristic of some of the Compositae (Roth 1977).

The cypselae of the species of *Senecio* and *Vernonia* all possessed duplex trichomes (Figures 22 and 23) (c/state 0). The remaining vestitured cypselae were devoid of duplex trichomes (c/state 1).

Types of duplex trichomes present (SEM Char. 11): The cypselae of the *Senecio* species (Figures 13–15) possessed finger-like duplex trichomes which were blunt-ended (c/state 0). Those of *V. poskeana* (Figure 22) and *V. oligocephala* (Figure 23) had forked duplex trichomes (c/state 2). The cypselae of *V. pauciflora* had narrow bristle-like duplex trichomes which were not forked at their tips (c/state 1).

Location of the duplex trichomes (SEM Char. 12): The trichomes of the *Senecio* species (Figures 13–15) were located in the furrows of the cypselae (c/state 0). Those of *V. poskeana* (Figure 22) were located only on the ribs (c/state 1). The duplex trichomes of *V. oligocephala* and *V. pauciflora* (Figures 23 and 24) were located over the entire surface of the cypselae (c/state 2).

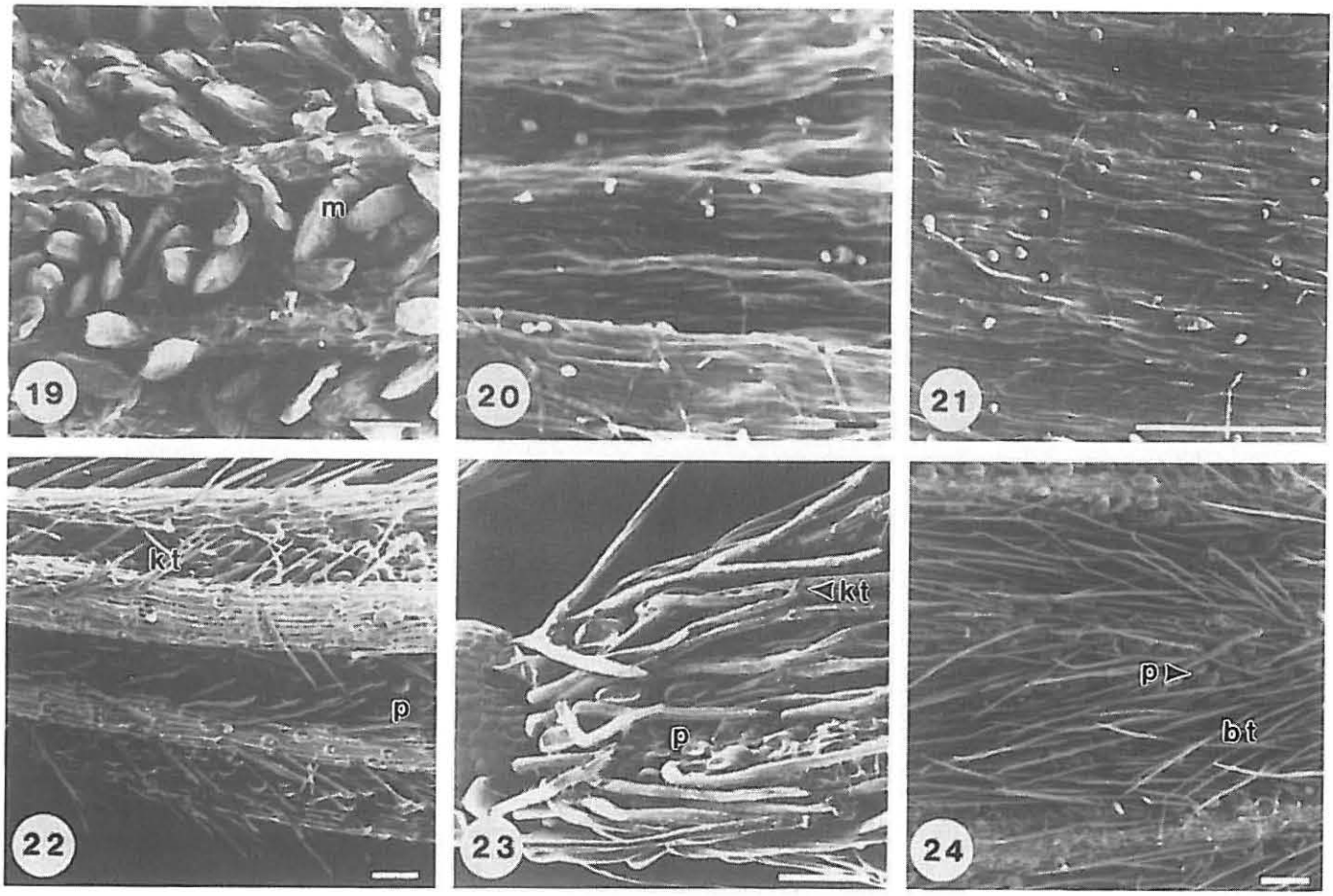
Presence or absence of small, spherical papillose cells (papillae) (SEM Char. 13): Small, spherical papillose cells (papillae) were found on the vestitured cypselae of the *Vernonia* species (Figures 22–24) (c/state 1), but were absent from the remaining taxa with vestitured cypselae (c/state 0). The papillae may contain mucilage or slime, which may serve to attach the fruit to the substratum, or they may represent a water storage tissue for the germinating embryo (Roth 1977).

Location of the papillae (SEM Char. 14): The papillae of *V. poskeana* (Figure 22) were restricted to the regions between the ribs (c/state 0), while those of *V. oligocephala* (Figure 23) were located on the ribs only (c/state 1). The papillae of *V. pauciflora* (Figure 24) were scattered over the entire cypsela surface amongst the trichomes (c/state 2).

Presence or absence of large, bulbous mucilaginous cells (SEM Char. 15): Large, bulbous mucilaginous cells were associated with the cypselae of *B. repanda* (Figure 18) and those of *B. nanum* (Figure 19) (c/state 1), but were absent from the remaining taxa with vestitured cypselae (c/state 0). These cells probably have the same or similar function(s) as those of the papillae (character 13).

Location of the large, bulbous mucilaginous cells (SEM Char. 16): The large, bulbous mucilaginous cells of cypselae of *B. repanda* (Figure 18) were located sparsely over the cypsela surface (c/state 0). Those of *B. nanum* (Figure 19) covered the entire cypsela surface (c/state 1).

Presence or absence of a carpopodium (SEM Char. 17): The abcission zone, where the cypsela is separated from the receptacle, may or may not show development on the cypsela itself, of a special structure presumably facilitating separation (Haque & Godward 1984). This structure has been termed the carpopodium. It has been suggested that this characteristic thickening provides a rigid structure which does not contract on drying, thus



Figures 19–24 Scanning electron micrographs of cypselae showing external micromorphology: **19**. *B. nanum* - cypsela covered with large, bulbous mucilaginous cells (**m**); **20**. *L. discolor* and **21**. *L. ovatum* - ribbed cypselae are glabrous; **22**. *V. poskeana* - forked trichomes (**kt**) on ridges and papillae (**p**) in furrows; **23**. *V. oligocephala* - forked trichomes (**kt**) cover cypsela surface and papillae (**p**) scattered in between; **24**. *V. pauciflora* - bristle-like trichomes (**bt**) cover cypsela surface and papillae (**p**) scattered in between. Scale bars: **19**, **21–24** = 0.1 mm; **20** = 0.01 mm.

leading to the development of tension in the thin-walled cells of the abscission layer, which renders abscission easier (Haque and Godward 1984).

The morphology of the carpopodium varies considerably among species of the Compositae. The presence or absence of the carpopodium and features of the carpopodium can be used as taxonomic characters (Haque & Godward 1984). Where there is no carpopodium, the cells at the abcission zone do not have thickened walls and entirely resemble other superficial cells of the cypsela (Haque & Godward 1984).

In the present study the cypselae of *S. vulgaris* (Figure 25), *B. nanum* (Figure 27) and both species of *Abrotanella* did not possess a carpopodium (c/state 0). The cypselae of the remaining taxa possessed carpopodia (c/state 1) which varied greatly in structure and shape (see SEM Characters 18–20).

Symmetry of the carpopodium (SEM Char. 18): In their study, Haque and Godward (1984) found that the carpopodia of certain species were asymmetrical, consisting of more cells at one side than at the other. In the present study, the carpopodia of the cypselae of the two species of *Liabum* (see Figure 30) were asymmetrical (c/state 1), while the remaining taxa possessed carpopodia which were symmetrical (c/state 0).

Number of rows of cells forming the carpopodium (SEM Char. 19): Variation was observed in the number of rows of cells (number of cell layers) forming the carpopodium amongst the taxa investigated. In *S. madagascariensis* (Figure 26) and *S. inaequidens* the carpopodium comprised of only one or two rows of cells (c/state 0) (not shown in the Figures presented). In *B. nanum* (Figure 27) and *L. ovatum* (Figure 30) the carpopodium comprised of three to five rows of cells (c/state 1). The carpopodium of *B. repanda* (Figure 28), *L. discolor* and the three species of *Vernonia* (see Figures 31 and 32) consisted of more than six rows of cells (c/state 2).

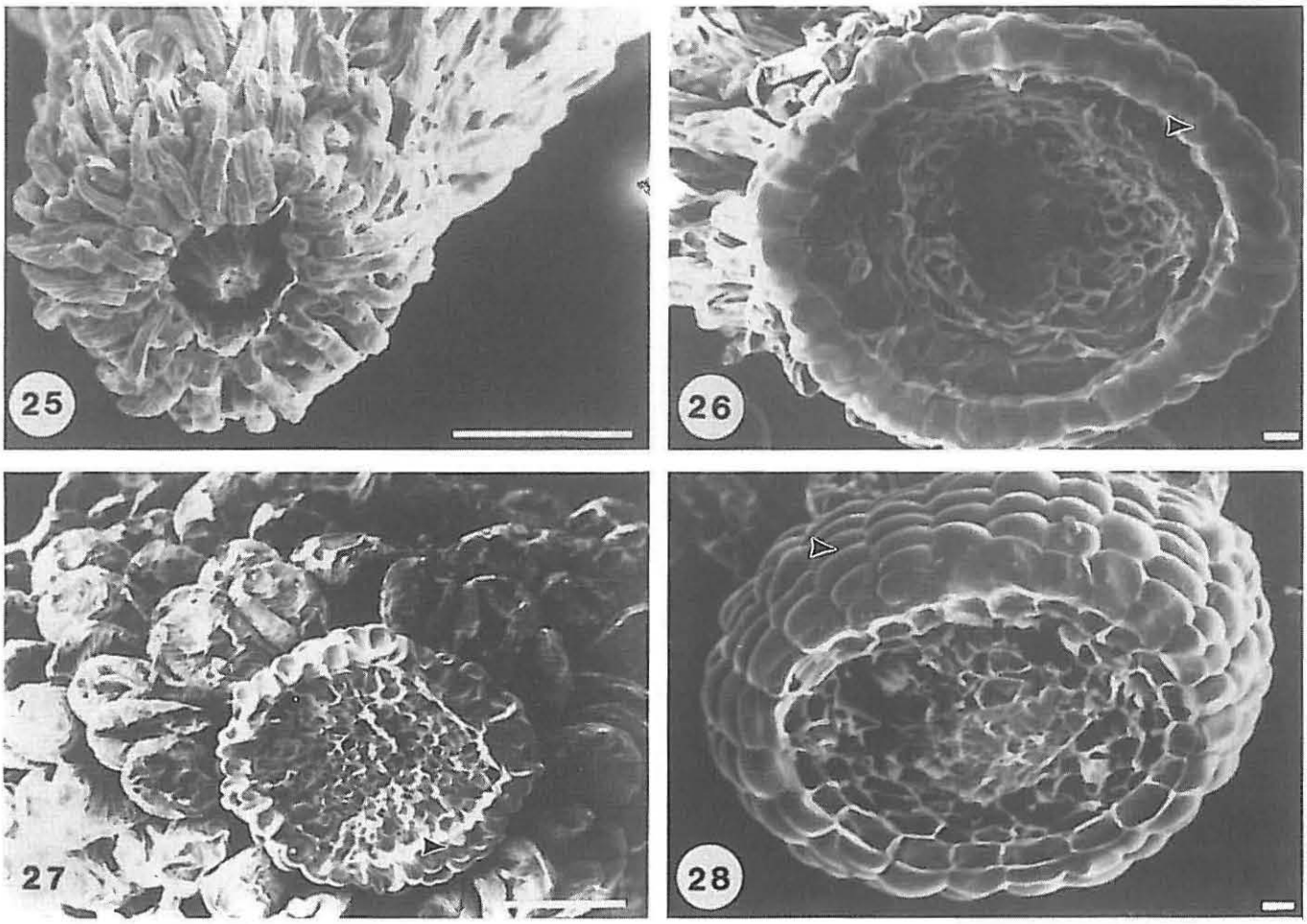
Cell outlines of the carpopodium (SEM Char. 20): Two character states were recognised. The carpopodium of *S. madagascariensis* (Figure 26), *S. inaequidens*, *B. nanum* (Figure 27), *B. repanda* (Figure 28) and *V. oligocephala* (Figure 31) had cell outlines which were distinctly visible (c/state 0). The cell outlines of the remaining taxa were only slightly visible (c/state 1).

Presence or absence of setose pappus (SEM Char. 21): A setose ('hairy') pappus was associated with most of the taxa investigated (c/state 0). *B. nanum*, *A. emarginata* and *A. scapigera* (all belonging to the subtribe Blennospermatinae) lacked a setose pappus (c/state 1).

Characters and character states of the cypsela micromorphology recorded via the light microscope (LM) study

Presence or absence of ribbing on the cypselae (LM Char. 1): The cypselae of *A. emarginata* lacked ribs (Figures 5 and 35) (c/state 0) while the cypselae of all of the remaining taxa possessed fibbing of some form (c/state 1).

Number of ribs on the cypsela surface (LM Char. 2): Three character states represent the variation in the number of ribs



Figures 25–28 Scanning electron micrographs of carpopodial region of the cypselae: **25**. *S. vulgaris* - no carpopodium present; **26**. *S. madagascariensis* symmetrical carpopodium (of 2 rows of cells when viewed from side) - cell outlines distinct; **27**. *B. nanum* - symmetrical carpopodium (of 3 rows of cells when viewed from side) - cell outlines distinct; **28.** *B. repanda* - symmetrical carpopodium of more than 6 rows of cells - cell outlines distinct. Scale bars: **25** and **27** = 0.1 mm; **26** and **28** = 0.01 mm.

formed on the surface of the cypselae. The cypselae of *B. repanda* possessed 5 ribs (c/state 1), while the cypselae of *B. nanum* (Figure 39) and *V. poskeana* (Figure 42) possessed 7 or 8 ribs (c/state 2). Ten or 11 ribs were present on the cypselae of *S. vulgaris* (Figure 33) and *L. ovatum* (Figure 44).

In the study on the development and structure of fruits in the tribe Vernonieae (Pandoy & Singh 1980) the number of cypsela ribs (ridges) varied, in some species, according to the placement of the florets in the capitulurn. The sampling strategy used in the present study did not allow for this variation to be investigated.

Nature of cypsela ribbing (LM Char. 3): This character describes the overall size of the ribs of each cypsela. The ribs of *L. ovatum* (Figure 37) comprised of alternating small and large ribs (c/state 2). Pandoy and Singh (1980) also observed such ribs in *V. anthelmintica* Willd. In the remaining taxa the ribs were the same size on each cypsela (c/state 1). The variation in the size of the ribs between the different taxa was not investigated in the present study.

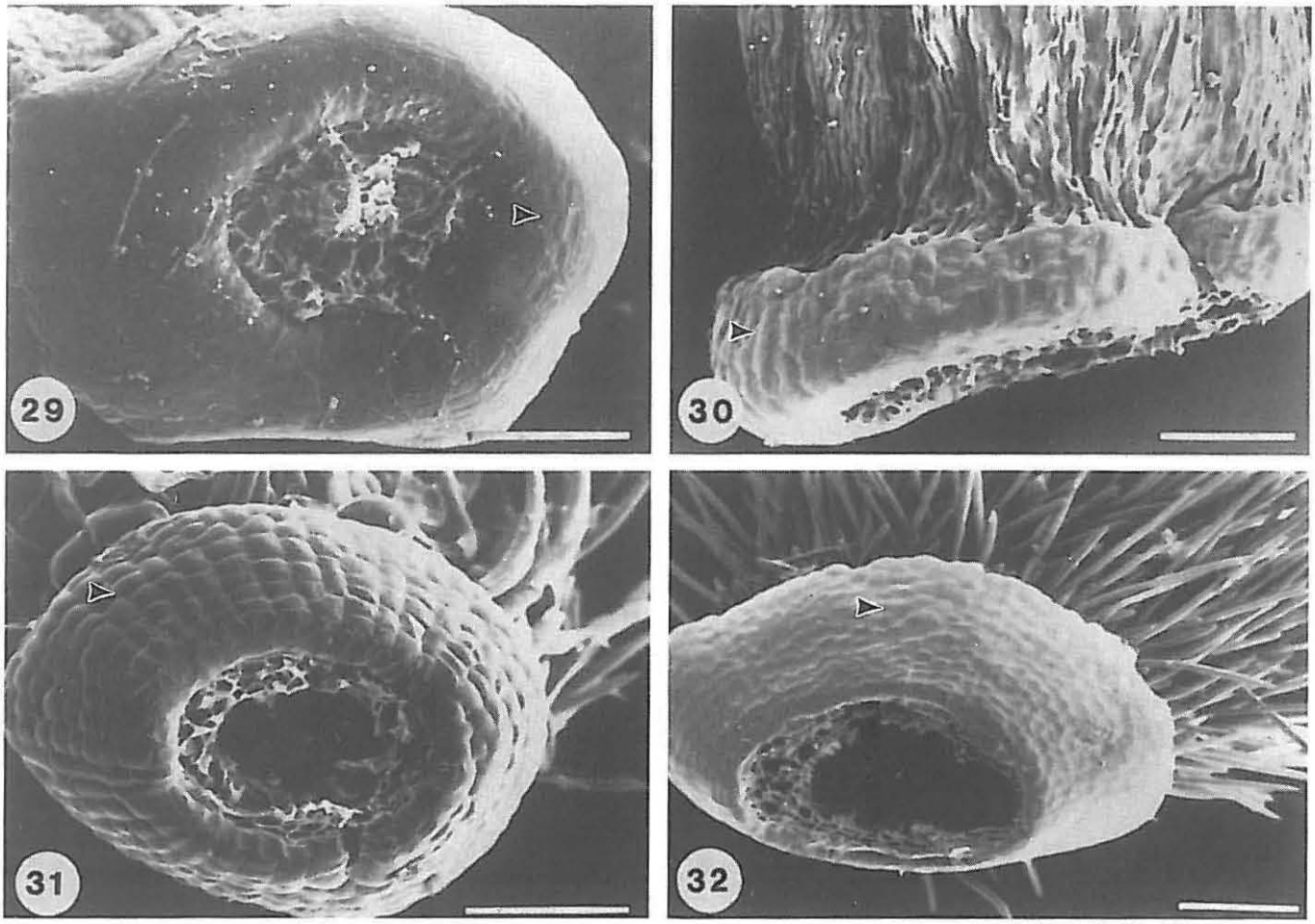
Orientation of pericarp vascular bundles (LM Char. 4): In those taxa in which pericarp vascular bundles were observed, the bundles were distinctly associated with the ribs of the cypselae (e.g. *B. repanda, B. nanum, L. ovatum* and *V. poskeana* (Figure 43) (c/state 1). The cypselae of *A. emarginata* were not ribbed and the four vascular bundles were evenly spaced around the perimeter of the pericarp (Figure 35) (c/state 2).

Presence or absence of lignified parenchyma in the mesocarp (LM Char. 5): Parenchyma forming the fruit wall may be one to several-layered and where it persists in the mature fruit it develops wall thickenings and may lignify. These parenchyma cells often show a special wall structure and are sometimes strengthened by reticulate, scalariform, ledge-shaped or pitted wall thickenings (Roth 1977).

The presence of lignified parenchyma was noted in the cypselae of *S. vulgaris* (Figure 34), *A. emarginata* (Figure 36), *L. ovatum* (Figure 38) *B. repanda* (Figure 41), *V. poskeana* (Figure 43) and *V. pauciflora* (Figure 44) (c/state 1). Lignified parenchyma was absent in the cypselae of *B. nanum* (Figure 40) (c/state 0).

Number of rows of lignified parenchyma cells (LM Char. 6): The lignified parenchyma of cypselae of *S. vulgaris* (Figure 34) and *A. emarginata* (Figure 36) consisted of one to two rows of cells. The lignified parenchyma of *L. ovatum* (Figure 37), *B. repanda* (Figure 41) and *V. pauciflora* (Figure 44) was multi-layered, consisting of three or more rows of cells. In *V. pauciflora* the lignified parenchyma formed an arc or crescent between the fibrous zone and the vascular tissue (Figure 44-**ap**).

Presence or absence of a hypodermis of sclerenchyma (LM Char. 7): Frequently, in dry fruits such as the cypsela, the hypodermis represents a continuous layer of lignified cells (sclerenchyma) with special wall thickenings of the reticulate or annular type or with pitted walls (Roth 1977). The capacity of the hypodermis to absorb water and to spread it homogeneously over the entire pericarp periphery, is of great importance for the softening



Figures 29–32 Scanning electron micrographs of carpopodial region of the cypselae: **29**. *L. discolor* - asymmetrical carpopodium (when viewed from side), multi-layered/rowed carpopodium (cell outlines only slightly visible); **30**. *L. ovatum* - asymmetrical carpopodium of 3–5 rows of cells, cell outlines only slightly visible; **31** *V. oligocephala* - symmetrical, multi layered carpopodium, cell outlines distinct; **32**. *V. pauciflora* symmetrical, multi-layered carpopodium, cell outlines only slightly distinct. Scale bar: 0.1 mm.

and soaking of the fruit and the supply of water to the embryo (Roth 1977), with the water absorption being facilitated by the duplex trichomes.

A hypodermis was observed in the cypselae of *B. nanum* (Figure 40-bh) and *V. poskeana* (Figure 43-**bh**) (c/state 1), but was absent form the cypselae of the remaining taxa (c/state 0).

Nature of the hypodermis (LM Char. 8): The hypodermis of the cypselae of *B. nanum* (Figure 40-bh) and *V. poskeana* (Figure 43-bh) consisted of 2 to 4 layers of cells forming a continuous band below the epidermis (banded hypodermis) (c/state 1). The hypodermis of cypselae of *V. pauciflora* (Figure 44-ah) consisted of arcs of sclerenchyma associated with the ribs (c/state 2).

Nature of the banded hypodermis (LM Char. 9): The band-shaped hypodermis of *B. nanum* (Figure 40) had more or less the same thickness all the way around the pericarp (c/state 1). That of *V. poskeana* (Figure 43) was thicker at the ridges (c/state 2). In the latter case, the sides of the ridges consisted of three to four rows of sclerenchyma cells, while the apex of the ridge consisted of only one row of sclerenchyma cells (Figure 43).

Presence of absence of fibrous zones of sclerenchyma (LM Char. 10): The mechanical tissue of the cypselae of the Asteraceae consists of sclereids or fibres or a combination of both (Roth 1977). Where the sclerenchyma is concentrated in certain regions, for example, associated with ribs of vascular bundles, these are arranged according to the symmetry of the fruit (Roth 1977).

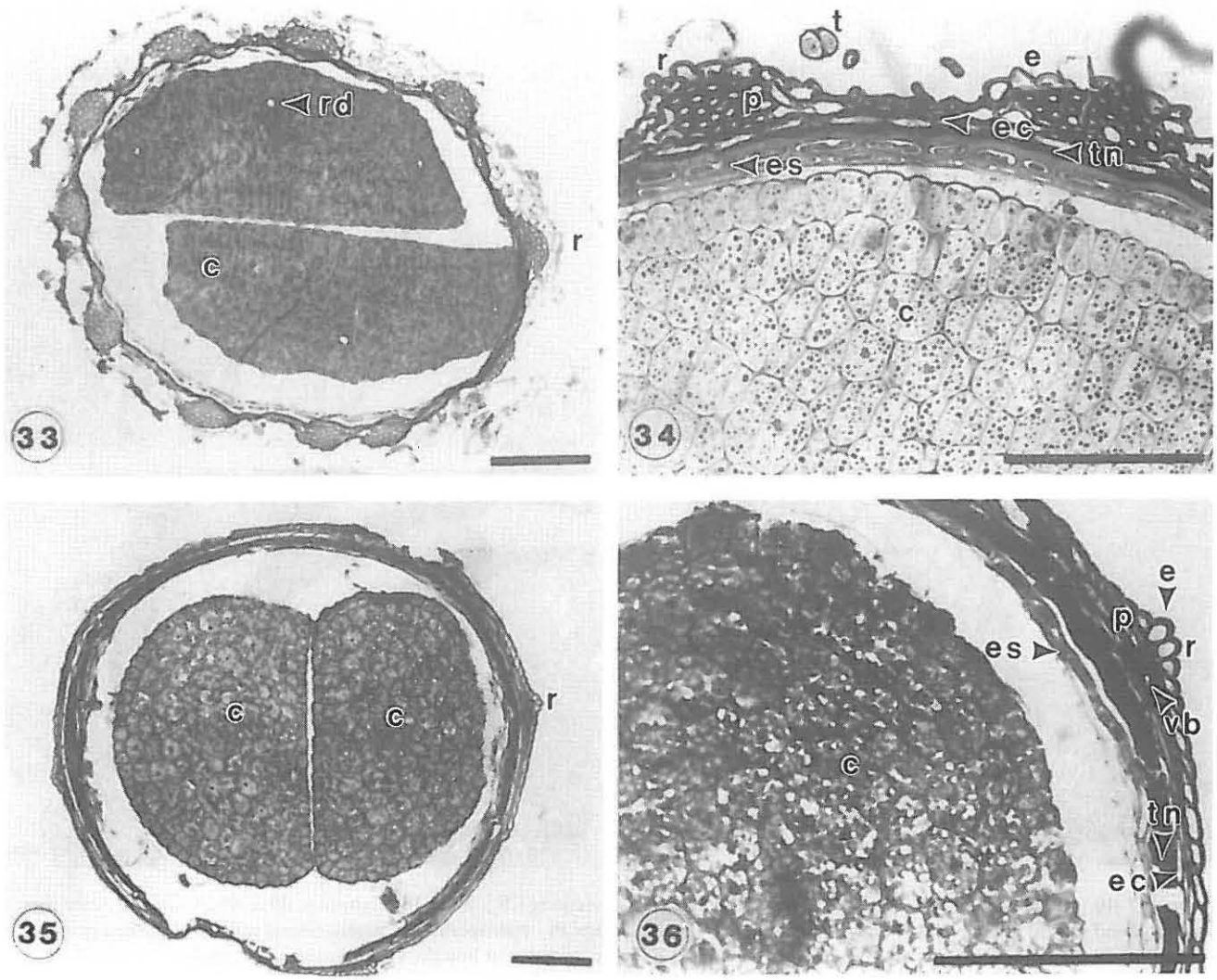
Such fibrous zones of sclerenchyma were observed in cross sections of the cypselae of *S. vulgaris* and *V. pauciflora* (Figure 44) (c/state 1), but were absent from the cypselae of the remaining taxa investigated (c/state 0).

Shape of the fibrous zones in transverse section (LM Char. 11): The fibrous zones of *S. vulgaris* (Figure 33) were spherical in shape in transverse section and were associated with the ribs of the fruit (c/state 1). The fibrous zones of *V. pauciflora* (Figure 44) were spherical and arc-shaped and also associated with the ribs of the fruit (c/state 2).

Presence or absence of resin ducts (LM Char. 12): Resin ducts, situated in the cotyledons, were only observed in the cypselae of *S. vulgaris* (Figure 33) (c/state 1) and were absent from the cypselae of the remaining taxa (c/state 0). However, cotyledons are not present in all the sections.

Phenetic Analyses - Cluster Analysis

The cluster analyses performed using the SEM, LM and the combined SEM and LM data sets, provided the following results. The UPGMA method produced the highest cophenetic correlation coefficient (r) values, using distance measures. The 'r' value for the SEM data was 0.94; for the LM data, r = 0.95 and for the combined SEM and LM data, r = 0.98. Consequently, the UPGMA method of clustering, using distance measures, was used for all the analyses. An 'r' value greater than 0.9 indicates a



Figures 33–36 Light micrographs of selected cypselae in transverse section. **33** & **34**. *S. vulgaris*; **35** & **36**. *A. emarginata*. Scale bars: 0.1 mm. (**c** - cotyledon; **e** - epidermis (epicarp); **ec** - endocarp; **es** - endosperm; **fz** fibrous zone; **p** - lignified parenchyma; **r** - rib; **rd** - resin duct; **t** - trichome in T.S.; **tn** - remains of testa and nucellus; **vb** - pericarp vascular bundle).

very good fit of the clustering matrix with the original matrix (Rohlf 1988). The other clustering methods used were: WPGMA, Flexible linkage, Complete linkage and Single linkage. In all cases the SAHN routine was run with the 'tie' option set to 'Find'. This was necessitated because more than one tree was found for each of the three data sets. The CONSENSUS option was used to produce a consensus tree, using the 'strict' setting, where multiple trees resulted from the cluster analyses using SAHN.

The phenogram based on the SEM data (Figure 45) is a consensus tree (strict) (based on 25 trees). It indicates relatively homogeneous intraspecific groupings with an acceptable amount of intraspecific variation. There is also distinct infrageneric grouping, forming homogenous generic clusters. The initial dichotomy is between members of Vernoneae and Senecioninae (*Senecio* and *Brachyglottis*), comprising one major cluster, and members of the Blennospermatinae (*Abrotanella* and *Blennosperma*) and Liabeae, which comprise the second major cluster.

The phenogram based on the LM data (Figure 46) is a consensus tree (strict) (based on 18 trees). It indicates that the representatives of the Vernonieae have a disjunct/dichotomous affinity to the other taxa. *V. pauciflora* has most affinity with *S. vulgaris* (Senecioninae), while *V. poskeana* has most affinity with *B. nanum* (Blennospermatinae) and then with *B. repanda* (Senecioninae) and *L. ovatum* (Liabeae). Note that the affinity of *A. emarginata* (Blennospermatinae) is basal and remote to the remaining taxa.

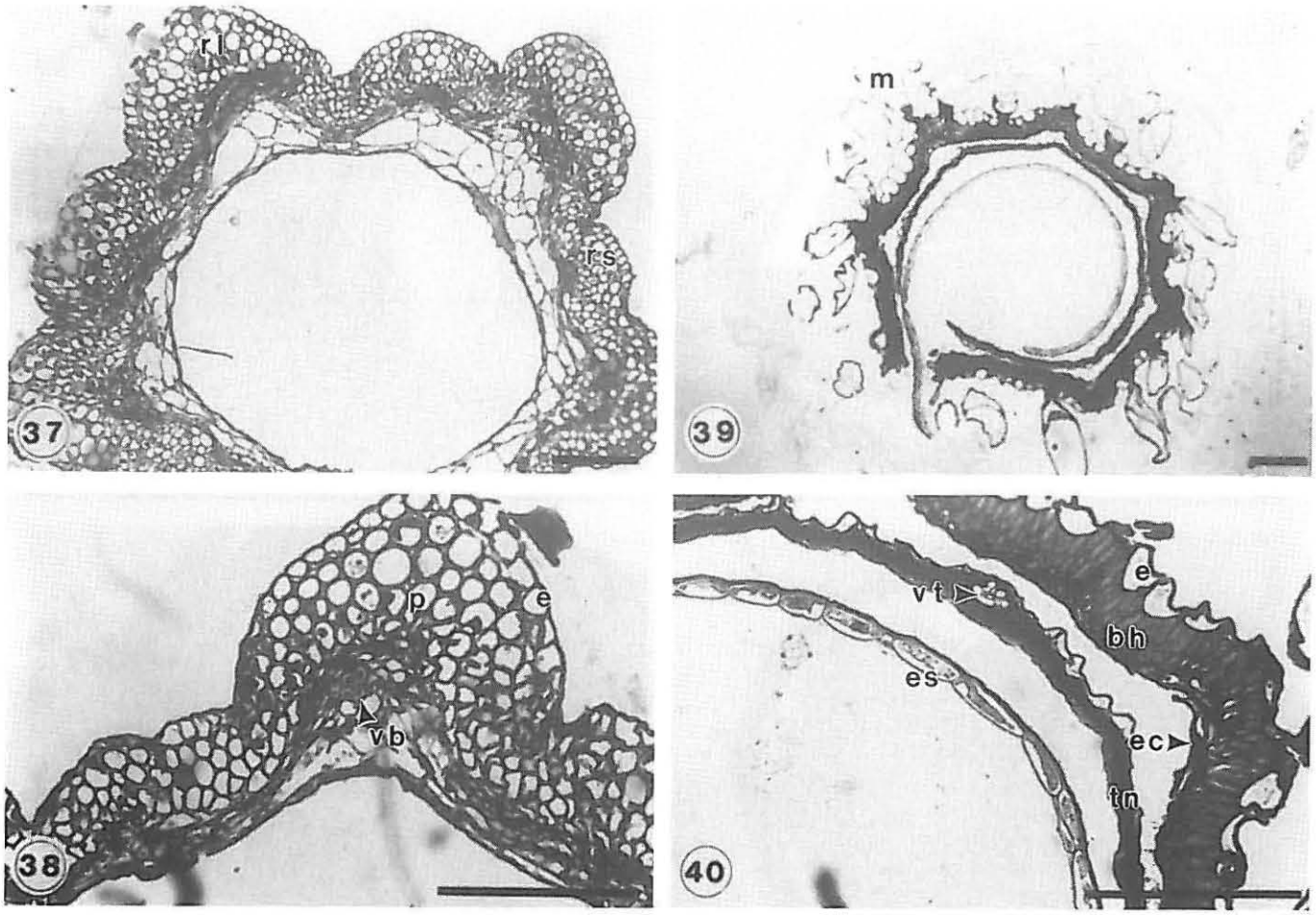
The phenogram based on the combined SEM and LM data (Figure 47) is a consensus tree (strict) (based on 3 trees). Note that the combined data matrix comprised only those taxa for which there was both SEM and LM data. This phenogram reflects phenetic relationships which are very similar to those based solely on the SEM data (Figure 45). A comparison with the phenogram based on the LM data (Figure 46) reveals both structural similarities and differences.

Principal Components Analyses (PCA)

PCA's were performed to obtain information on which characters are statistically important with regard to defining the relationships amongst the taxa in each of the analyses.

Principal Components Analysis (PCA) of SEM data

The PCA of the 21 SEM characters indicated which of these characters are most important in defining the relationships between the taxa (Figure 48). The first three components accounted for 72.3% of the variation in the data. **Component 1** had correlations greater than 0.5 (as indicated by the absolute values of the eigenvectors for the characters) for the characters



Figures 37–40 Light micrographs of selected cypselae in transverse section (T.S.): **37** & **38.** *L. ovatum*; **39** & **40**. *B. nanum*. Scale bars: 0.1 mm. (**bh** - band shaped hypodermis; **e** - epidermis (epicarp); **ec** - endocarp; **es** - endosperm; **m** - mucilaginous cells; **p** - lignified parenchyma; **rl** - large rib; **rs** - small rib; **tn** - remains of testa and nucellus; **vb** - pericarp vascular bundle; **vt** - testa vascular bundle).

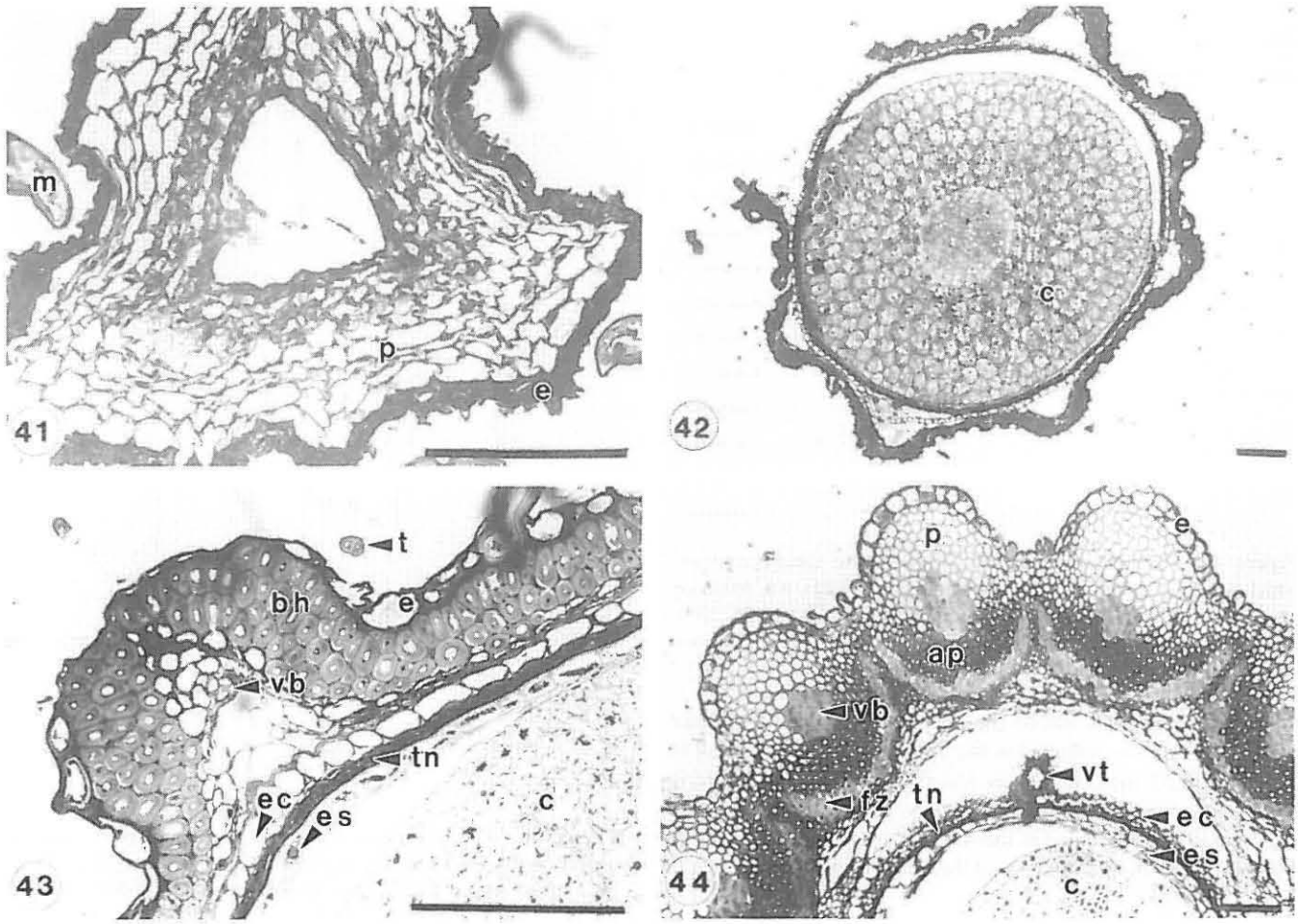
(in decreasing order of importance): Location of the verrucae (SEM Char. 9), Types of duplex trichomes present (SEM Char. 11), Shape of cypselae (SEM Char. 1), Length:Width ratio of cypselae (SEM Char. 2), Presence or absence of verrucae (SEM Char. 7), Location of the duplex trichomes (SEM Char. 12), Number of rows of cells forming the carpopodium (SEM Char. 19) and Presence or absence of duplex trichomes (SEM Char. 10). **Component 2** had correlations greater than 0.5 for the characters (in decreasing order of importance): Presence or absence of a setose pappus (SEM Char. 21), Location of the large, bulbous mucilaginous cells (SEM Char. 16), Number of different types of cells/trichomes forming the vestiture (SEM Char. 6) and Presence or absence of papillae (SEM Char. 13). **Component 3** had correlations greater than 0.5 for the characters (in decreasing order of importance): Presence or absence of vestiture (SEM Char. 5), Symmetry of the carpopoclium (SEM Char. 18), Cell outlines of the carpopodium (SEM Char. 20), Presence or absence of large, bulbous mucilaginous cells (SEM Char. 15) and Nature of ribbing (SEM Char. 4).

Principal Components Analysis (PCA) of LM data

In the PCA of the 12 LM characters the first three components accounted for 80.9% of the variation in the data (Figure 49). **Component 1** had correlations greater than 0.5 (as indicated by the absolute values of the eigenvectors for the characters) for the characters (in decreasing order of importance): Presence or absence of resin ducts (LM Char. 12), Presence or absence of a hypodermis of sclerenchyma (LM Char. 7), Presence or absence of lignified parenchyma in the mesocarp (LM Char. 5), Number of ribs formed on the fruit surface (LM Char. 2), Presence or absence of fibrous zones of sclerenchyma (LM Char. 10), Number of rows of lignified parenchyma cells present (LM Char. 6) and Shape of the fibrous zones in transverse section (LM Char. 11). **Component 2** had correlations greater than 0.5 for the character: Nature of the hypodermic (LM Char. 8). **Component 3** had correlations greater than 0.5 for the characters (in decreasing order of importance): Presence or absence of ribbing on the cypselae (LM Char. 1) and Orientation of pericarp vascular bundles (LM Char. 4).

Principal Components Analysis (PCA) of SEM and LM Data

In the PCA of the 33 characters from the LM and SEM components of the study the first three components accounted for 75.4% of the variation in the data (Figure 50). Note that this 75.4% for the value of the trace is in between the traces obtained for the PCA of the SEM data (72.3%) and the LM data (80.9%). **Component 1** had correlations greater than 0.5 (as indicated by the absolute values of the eigenvectors for the characters) for the characters (in decreasing order of importance): Types of duplex trichomes present (SEM Char. 11), Nature of the verrucae (SEM Char. 8), Shape of the cypselae (SEM Char. 1), Presence or absence of resin ducts (LM Char. 12), Location of the large, bulbous mucilaginous cells (SEM Char. 16), Presence or absence of duplex trichomes (SEM Char. 10), Length:Width ratio of cypselae (SEM Char. 2), Number of rows of lignified parenchyma cells present (LM Char. 6), Shape of the fibrous zones in transverse section (LM Char. 11), Presence or absence of a



Figures 41–44 Light micrographs of selected cypselae in transverse section (T.S.): **41**. *B. repanda*, **42** & **43**. *V. poskeana*; **44**. *V. pauciflora*. Scale bars: 0.1 mm. (**ap** - arc-shaped parenchyma; **bh** - band-shaped hypodermis; **c** - cotyledon; **e** - epidermis; **ec**- endocarp; **es** - endosperm; **fz** - fibrous zone; **m** - mucilaginous cell; **p** - lignified parenchyma; **t** - trichome in T.S. ; **tn** remains of testa and nucellus; **vb** - pericarp vascular bundle; **vt** - testa vascular bundle).

hypodermis of sclerenchyma (LM Char. 7), Presence or absence of verrucae (SEM Char. 7) and Presence or absence of fibrous zones of sclerenchyma (LM Char. 10). **Component 2** had correlations greater than 0.5 for the characters (in decreasing order of importance): Nature of the banded hypodermis (LM Char. 9), Location of the verrucae (SEM Char. 9), Location of the duplex trichomes (SEM Char. 12), Cell outlines of the carpopodium (SEM Char. 20), Symmetry of the carpopodium (SEM Char. 18), Presence or absence of vestiture (SEM Char. 5), Number of different types of cells/trichomes forming the vestiture (SEM Char. 6), Orientation of the pericarp vascular bundles (LM Char. 4), Number of rows of cells forming the carpopodium (SEM Char. 19) and Presence or absence of ribbing on the cypselae (LM Char. 1). **Component 3** had correlations greater than 0.5 for the characters (in decreasing order of importance): Nature of the banded hypodermis (LM Char. 9), Presence or absence of papillae (SEM Char. 13), Number of ribs formed on the fruit surface (LM Char. 2) and Presence or absence of lignified parenchyma in the mesocarp (LM Char. 5).

Note that the PCA based on the combined LM and SEM data (Figure 50) reflects relationships amongst the taxa which are similar to those produced using only the SEM data (Figure 48).

Discussion

Cluster analysis of the SEM and the combined LM and SEM data sets produced biologically meaningful phenograms (Figures 45 and 47), with the analysis of the SEM data producing the more meaningful phenogram. The phenogram based on the LM data (Figure 46), was not as biologically meaningful, the clustering pattern differing considerably from that produced using the SEM data. This is probably due to the LM data set comprising relatively few characters which described the anatomy of the cypselae. Furthermore, a number of characters could not be recorded

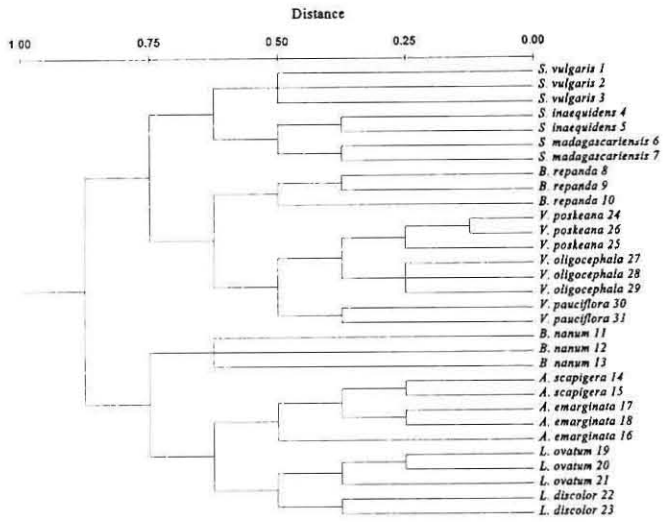


Figure 45 Phenogram (using UPGMA) of all the specimens investigated (Appendix 1) based on the SEM data (Appendix 4) obtained from the 21 micromorphological characters (Appendix 2) studied. Cophenetic correlation coefficient (r) = 0.94. Note that this is a strict consensus tree, based on 25 trees. The specimen number follows the species name.

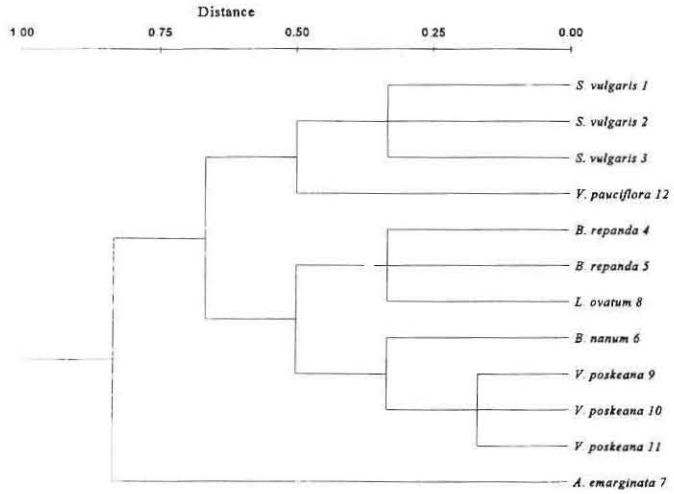


Figure 46 Phenogram (using UPGMA) of the specimens from which anatomical data, using Light Microscopy (LM), was obtained (Appendix 5). Cophenetic correlation coefficient (r) = 0.95. Note that this is a strict consensus tree, based on 18 trees. The specimen number follows the species name

for certain specimens due to the poor quality of the herbarium material which was reflected in the anatomical preparations. The combined SEM and LM data set was also not fully representative of all the taxa included in the SEM study. Biological meaningfulness should be understood in the sense of coincidence with existing overall systematic opinion on the affinity between the taxa in question.

In the phenogram based on the SEM data (Figure 45) members of *Senecio* were most similar to the species of *B. repanda*, in terms of distance. This was anticipated considering their current subtribal affinity.

The phenograms based on the SEM data (Figure 45) and the combined LM and SEM data (Figure 47) produced similar and biologically meaningful clusters with a noticeable measure of infrageneric clustering for all the genera investigated. This is clearly depicted in the phenogram based on the SEM data (Figure 46). However, the position of the species of *Abrotanella* and *Blennosperma* (subtribe Blennospermatinae) were obscure. The

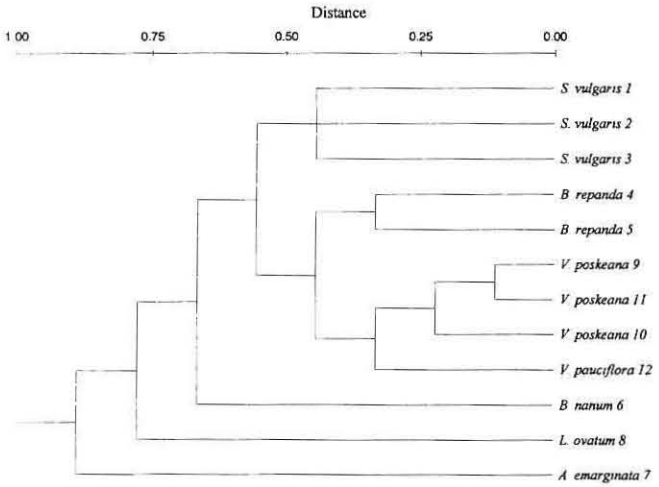


Figure 47 Phenogram (using UPGMA) of the specimens from which both micromorphological (SEM) and anatomical (LM) data (33 characters) was obtained. Cophenetic correlation coefficient (r) = 0.98. Note that this is a strict consensus tree, based on 3 trees. The specimen number follows the species name

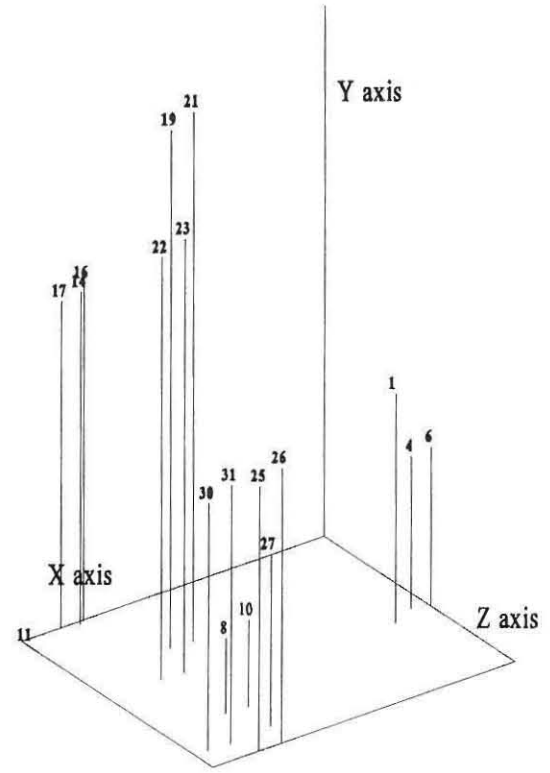


Figure 48 Plot of the first three principal component axes from analysing all the taxa investigated (Appendix 1), with respect to data obtained for the 21 micromorphological (SEM) characters investigated. Each numbered point represents 1–3 specimens of the various species investigated. The coincident specimen(s) can be determined by consulting the relevant phenogram

fruits of these genera were very different from those of the other taxa studied, including those of the Senecioninae.

The phenograms based on SEM data (Figure 45) and SEM and LM data (Figure 47) both indicate an affinity between *Abrotanella* (Blennospermatinae) and *Liabum* (Liabeae), with

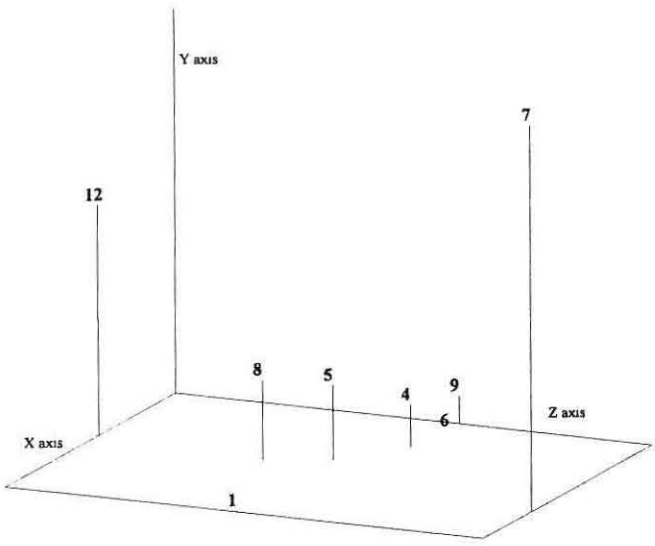


Figure 49 Plot of the first three principal component axes from analysing all the taxa from which the LM data of the 12 anatomical characters were obtained (Appendix 3). Each numbered point represents 1–3 specimens of the various species investigated. The coincident specimen(s) can be determined by consulting the relevant phenogram.

variable affinity with *B. nanum* (Blennospermatinae). Taxonomically the expected affinity would have been between *Abrotanella* and *B. nanum*. The fact that *Abrotanella* and *Blennosperma* are associated with the lower branch of the dichotomous phenogram is taxonomically significant. This conflict of affinity is acceptable as the subtribe Blennospermatinae is considered to be a discordant member of the Senecioneae and is included in the Senecioneae with some reluctance (Nordenstam 1977; Bremer 1994, p.481). Furthermore, the taxonomic placement of the Blennospermatinae is a somewhat tentative solution and perhaps peripheral to the much larger taxonomic problems regarding the composition of the Senecioneae (Bremer 1994, p. 481). The obscure position of the species of *Abrotanella* and *Blennosperma*, cited above, is therefore perhaps to be expected.

The three-dimensional PCA plots of the taxa of the SEM and the combined LM and SEM data sets (Figures 48 and 50) produced similar groupings as those produced in the cluster analyses.

The PCA analyses produced valuable information on which characters are statistically important with respect to defining the phenetic relationships amongst the taxa studied. A large number of the SEM and LM characters have correlations greater than 0.5% in the PCA analyses. This indicates the importance of many of the SEM and LM characters recorded in recognising discontinuities between the taxa studied. It could be argued that this significance is restricted to the taxa in question. However, it is argued that the type and range of characters investigated in this study would assist other synantherological studies. Considering the analysis of the combined SEM and LM data, some of both the SEM and the LM characters are statistically important in elucidating phenetic relationships. This finding is implicitly important for it indicates that data obtained from either or both sources is taxonomically useful.

This investigation clearly supports the useful role of the characters of surface micromorphology and anatomy of the cypselae in recognising biological and taxonomic discontinuities amongst the taxa investigated and, by extrapolation, in the Asteraceae.

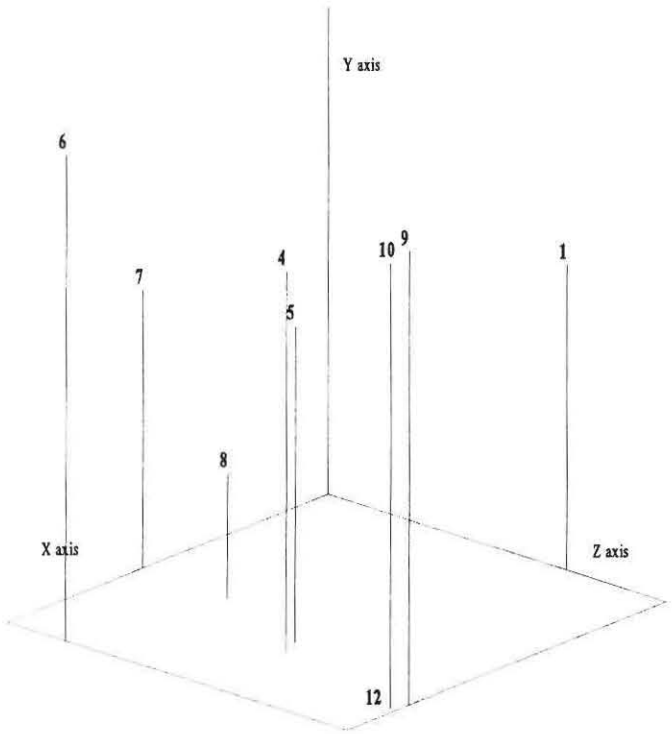


Figure 50 Plot of the first three principal component axes from analysing all the taxa from which data on both the 21 micromorphological (SEM) and the 12 anatomical (LM) characters, was obtained. Each numbered point represents 1–3 specimens of the various species investigated. The coincident specimen(s) can be determined by consulting the relevant phenogram.

Acknowledgements

The authors thank the University Research Committee of the University of the Witwatersrand for financial assistance to conduct this research.

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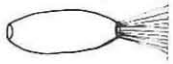
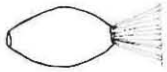
Appendix 1 List of specimens of each species investigated in this study, with an indication of the tribal/subtribal affinity

Spec. No	Taxon	Collector(s)	Coll. no.	Locality	Herbarium	SEM &/or LM
Tribe Senecioneae subtribe Senecioniae						
1	*Senecio vulgsris* L. (tyoe of genus)	Morrison	s.n.	Labrador, N. America	K	SEM & LM
2	ditto	Tateishi & Ohba	s.n.	Miyagi, Japan	K	SEM & LM
3	ditto	G.B. Hinton	8327	Crucero, S.. America	K	SEM & LM
4	*Senecio inaequidens* DC.	Hilliard & Burtt	10923	Hogsback, S. Africa	K	SEM
5	ditto	M.J.A. Wagner	863	Augrabies, S. Africa	PRE	SEM
6	*Senecio madagascariensis* Poir.	O. West	1128	Estcourt, S. Africa	K	SEM
7	ditto	R.K. Grosvenor	162	Umhlanga Rocks, S. Africa	PRE	SEM
8	*Brachyglottis repanda* J.R. & G. Forst. (type of subtribe)	R.C. Cooper	s.n. (K750479)	Bay of Islands County, New Zealand	K	SEM & LM
9	ditto	R. Melville & L.B. Moore	5199	North Island, New Zealand	K	SEM & LM
10	ditto	A.W. Anderson	175	South Island, New Zealand	K	SEM
Tribe Senecioneae subtribe Blennospermatinae						
11	*Blennosperma nanum* (Hook.) Blake ssp. *nanum*	J.T. Howell	4144	Mercedo County, California	K	SEM
12	ditto	C.G. Pringle	s.n.	Wilmington, California	K	SEM
13	ditto	J.T. Howell	1753	Soscol, Napa County, California	K	SEM & LM
14	*Abrotanella scapigera* F. Muell. (type of subtribe)	G. Lopez	s.n.	Cradle Mtns, Tasmania	K	SEM
15	ditto	G. Lopez	s.n.	ditto	K	SEM
16	*A. emarginata* Cass.	Hooker	44	Falkland Islands	K	SEM
17	ditto	S.W. Greene	82/1	Port Stanley, Falkland Islands	K	SEM & LM
18	ditto	S.W. Greene	85/1	ditto	K	SEM
Tribe Liabeae						
19	*Liabum ovatum* (Wedd.) Ball. (type of tribe)	S.G.E. Saunders & San Marcus	1093	Province of Concepçion, Peru	K	SEM & LM
20	ditto	A. & P. McKlean	s.n.	Not provided - presumably S. America	K	SEM
21	ditto	W.J. Eyerdam	25060	Chocamba, Bolivia	K	SEM
22	*L. discolor* Hemsl.	H. von Tuerckheim	8412	Alta Verapaz, Guatemala	K	SEM
23	ditto	J.D.G.	2355	Guatemala	K	SEM
Tribe Vernoneae						
24	*Vernonia poskeana* var. *centauroides* (Klatt.) Wild. (*V. poskeana* = type of genus)	J. Borle	530	Lourenço Marques (Mozambique)	PRE	SEM & LM
25	*Vernonia poskeana* var. *poskeana*	L.E. Codd	2143	Baortseland, N. Rhodesia (Zambia)	PRE	SEM & LM
26	*Vernonia poskeana* var. *centauroides*	G. Pedro	83	Lourenço Marques (Mozambique)	PRE	SEM & LM
27	*V. oligocephala* (DC.) Sch.BIP.	O. Bär	30033	Omanbondotal, S.W.A. (Namibia)	PRE	SEM
28	ditto	L.E. Codd	s.n.	Pretoria University Farm (South Africa)	PRE	SEM
29	ditto	O.J. Hansen	3194	Gaberone, Botswana	PRE	SEM
30	*V. pauciflora* Less.	I.H. Patel & N. Nachamba	2238	Lifidzi Breeding Centre, Malawi	PRE	SEM & LM
31	ditto	J.G. Adams	15873	Hiokal Kaba Badi Village, Senegal	PRE	SEM

Appendix 2 List of the 21 micromorphological characters and their character states used in the scanning electron microscope (SEM) component of this study

Character 1: Shape of cypsela

1  Cypsela narrowly elliptic in shape, being oval in outline and narrowed towards its ends and widest at or about the middle (= fusiform in shape)

2  Cypsela elliptic in shape, being broader at its widest point than in character state 1

3  Cypsela narrowly obovate in shape, with the distal half broader than the proximal half

4  Cypsela obovate in shape, being broader than that in character state 3

5  Cypsela ovate in shape, being broader at its proximal half than at its distal half

Character 2: Length:Width ratio of cypsela

1 Ratio between 1.9 and 3.0

2 Ratio between 3.1 and 3.9

3 Ratio between 4.8 and 7.2

Character 3: Presence or absence of ribbing on the cypsela

0 Ribbing present

1 Ribbing absent

Character 4: Nature of ribbing

0  Ribs of equal size

1  Alternating small and large ribs

Character 5: Presence or absence of vestiture

0 Vestiture of some sort present

1 Vestiture absent

Character 6: Number of different types of cells/trichomes forming the vestiture

0 2 types of cells/trichomes present

1 1 type of cell/trichome present

Character 7 Presence or absence of verrucae

0 Verrucae present

1 Verrucae absent

Character 8: Nature of the verrucae

0  Verrucae with blunt ends

1  Verrucae pointed at their distal ends

Character 9: Location of the verrucae

0 Verrucae located only on the ridges

1 Verrucae cover the entire fruit surface

Character 10: Presence or absence of duplex trichomes

0 Duplex trichomes present

1 Duplex trichomes absent

Appendix 2 Continued

Character 11: Types of duplex trichomes present

0  Finger-like duplex trichomes

1  Bristle-like duplex trichomes

2  Forked duplex trichomes

Character 12: Location of the duplex trichomes

0 Located only in the furrows on the surface of the cypsela

1 Located only on the ridges on the surface of the cypsela

2 Covering the entire surface of the cypsela

Character 13: Presence or absence of small, spherical papillose cells (papillae)

0 Papillae absent

1  Papillae present

Character 14: Location of the papillae

0 Papillae located only in the furrows on the surface of the cypsela

1 Papillae located only on the ridges on the surface of the cypsela

2 Papillae scattered over the entire cypsela surface, in between the trichome

Character 15: Presence or absence of large, bulbous mucilaginous cells

0 Mucilaginous cells absent

1  Mucilaginous cells present

Character 16: Location of large, bulbous mucilaginous cells

0 Mucilaginous cells located sparsely over the cypsela surface

1 Mucilaginous cells covering the entire cypsela surface

Character 17: Presence or absence of a carpopodium

0 Carpopodium absent

1 Carpopodium present

Charater 18: Symmetry of the carpopodium

0  Carpopodium symmetrical

1  Carpopodium asymmetrical

Character 19: Number of rows of cells forming the carpopodium

0 1 to 2 rows of cells

1 3 to 5 rows of cells

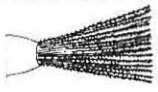
2 More than 6 rows of cells

Character 20: Carpopodium cell outlines

0 Cell outlines distinctly visible

1 Cell outlines only slightly visible

Character 21: Presence or absence of a setose pappus

0  Setose pappus present

1 Setose pappus absent

Appendix 3 List of the 12 anatomical characters and their character states used in the light microscope (LM) component of the study

Character 1: Presence or absence of ribbing on the cypsela	
0	Ribbing absent
1	Ribbing pressent
Character 2: Number of ribs formed on the friut surface	
1	5 ribs present
2	7–8 ribs present
3	10–11 ribs present
Character 3: Nature of ribbing	
1	Ribs of equal size
2	Alternating large and small ribs
Character 4: Orientation of pericarp vascular bundles	
1	Vascular bundle associated with each rib
2	Vascular bundles evenly spaced around the circumference of the pericarp
Character 5: Presence or absence of lignified parenchyma in the mesocarp	
0	Parenchyma absent
1	Parenchyma present
Character 6: Number of rows of lignified parenchyma cells present	
1	1–2 rows of cells present
2	Parenchyma multi-layered, of 3 or more rows of cells
Character 7: Presence or absence of a hypodermis of sclerenchyma	
0	Hypodermis absent
1	Hypodermis present
Character 8: Nature of the hypodermis	
1	Hypodermis forms a band around the circumference of the cypsela
2	Hypodermis consists of arcs of sclerenchyma associated with the ribs
Character 9: Nature of the banded hypodermis	
1	Hypodermis of more or less the same thickness all the way round the cypsela in T.S.
2	Hypodermis thicker at the ribs of the cypsela in T.S.
Character 10: Presence or absence of fibrous zones of sclerenchyma	
0	Fibrous zones absent
1	Fibrous zones present
Character 11: Shape of fibrous zones in T.S.	
1	Fibrous zones spherical in shape
2	Fibrous zones spherical and arc-shaped
Character 12: Presence or absence of resin ducts	
0	Resin ducts absent
1	Resin ducts present

Appendix 4 Data matrix of the 21 micromorphological characters of the cypselae (Appendix 2) obtained from the Scanning Electron microscopy (SEM) studies. The numerical values in the matrix correspond to the relevant character stated of the character concerned (see Appendix 2 for details). The specimen numbers in the first column are for administrative purposes only. A '-' indicates an inapplicable character for the species concerned (missing data). In the phenetic analyses, missing data was denoted by the number 9. The actual Length:Width ratios for character 2 have been included in the matrix as character 2.1. These data were not included in the phenetic analyses

Spec.		Characters																					
No.	Species	1	2	2.1	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	*Senecio vulgaris*	1	3	6.23	0	0	0	0	0	0	0	0	0	0	0	-	0	-	0	-	-	-	0
2	ditto	1	3	5.61	0	0	0	0	0	0	0	0	0	0	0	-	0	-	0	-	-	-	0
3	ditto	1	3	5.24	0	0	0	0	0	0	0	0	0	0	0	-	0	-	0	-	-	-	0
4	*Senecio maequadens*	2	3	5.76	0	0	0	0	0	1	0	0	0	0	0	-	0	-	1	0	0	0	0
5	ditto	2	3	7.24	0	0	0	0	0	1	0	0	0	0	0	-	0	-	1	0	0	0	0
6	*Senecio madagascariensis*	1	3	5.04	0	0	0	0	0	1	0	0	0	0	0	-	0	-	1	0	0	0	0
7	ditto	1	3	5.27	0	0	0	0	0	1	0	0	0	0	0	-	0	-	1	0	0	0	0
8	*Brachyglottis repanda*	3	1	2.91	0	0	0	0	0	0	1	1	-	-	0	-	1	0	1	0	2	0	0
9	ditto	3	1	2.79	0	0	0	0	0	0	1	1	-	-	0	-	1	0	1	0	2	0	0
10	ditto	3	2	3.24	0	0	0	0	0	0	1	1	-	-	0	-	1	0	1	0	2	0	0
11	*Blennosperma nanum* ssp. *nanum*	5	2	2.60	0	0	0	1	1	-	-	1	-	-	0	-	1	1	1	0	1	0	1
12	ditto	5	2	2.58	0	0	0	1	1	-	-	1	-	-	0	-	1	1	1	0	1	0	1
13	ditto	5	2	2.44	0	0	0	1	1	-	-	1	-	-	0	-	1	1	1	0	1	0	1
14	*Abrotanella scapigera*	3	1	2.32	1	-	1	-	1	-	-	1	-	-	0	-	0	-	0	-	-	-	1
15	ditto	3	1	3.08	1	-	1	-	1	-	-	1	-	-	0	-	0	-	0	-	-	-	1
16	*A. emarginata*	4	2	3.25	1	-	1	-	1	-	-	1	-	-	0	-	0	-	0	-	-	-	1
17	ditto	4	1	2.30	1	-	1	-	1	-	-	1	-	-	0	-	0	-	0	-	-	-	1
18	ditto	4	1	2.78	1	-	1	-	1	-	-	1	-	-	0	-	0	-	0	-	-	-	1
19	*Liabum ovatum*	3	2	3.56	0	1	1	-	1	-	-	1	-	-	0	-	0	-	1	1	1	1	0
20	ditto	3	2	3.85	0	1	1	-	1	-	-	1	-	-	0	-	0	-	1	1	1	1	0
21	ditto	3	3	4.85	0	1	1	-	1	-	-	1	-	-	0	-	0	-	1	1	1	1	0
22	*L. discolor*	3	1	2.26	0	0	1	-	1	-	-	1	-	-	0	-	0	-	1	1	2	1	0
23	ditto	3	2	3.30	0	0	1	-	1	-	-	1	-	-	0	-	0	-	1	1	2	1	0
24	*Vernonia poskeana* var. *centauroides*	3	2	3.39	0	0	0	0	1	-	-	0	2	1	1	0	0	-	1	0	2	1	0
25	*Vernonia poskeana* var. *poskeana*	3	1	3.04	0	0	0	0	1	-	-	0	2	1	1	0	0	-	1	0	2	1	0
26	*Vernonia poskeana* var. *centauroides*	3	2	3.92	0	0	0	0	1	-	-	0	2	1	1	0	0	-	1	0	2	1	0
27	*V. oligocephala*	3	1	1.93	0	0	0	0	1	-	-	0	2	0	1	1	0	-	1	0	2	0	0
28	ditto	3	1	2.64	0	0	0	0	1	-	-	0	2	0	1	1	0	-	1	0	2	0	0
29	ditto	3	1	1.96	0	0	0	0	1	-	-	0	2	0	1	1	0	-	1	0	2	0	0
30	*V. pauciflora*	5	1	3.00	0	0	0	0	1	-	-	0	1	2	1	2	0	-	1	0	2	1	0
31	ditto	5	2	3.38	0	0	0	0	1	-	-	0	1	2	1	2	0	-	1	0	2	1	0

Appendix 5 Data matrix of the 12 anatomical characters of the cypselae (Appendix 3) obtained from the Light Microscopy (LM) studies. The numerical values in the matrix correspond to the relevant character states of the character concerned (see Appendix 3 for details). The specimen numbers in the first column are for administrative purposes only. A '?' indicates missing data due to the poor quality of the herbarium material. A '-' indicates an inapplicable character for the species concerned. In the martix used for the phenetic analyses, missing data (- & ?) was denoted by the number 9

		Characters											
Spec. No.	Species	1	2	3	4	5	6	7	8	9	10	11	12
1	*Senecio vulgaris*	1	3	1	?	1	1	0	-	-	1	1	1
2	ditto	1	3	1	?	1	1	0	-	-	1	1	1
3	ditto	1	3	1	?	1	1	0	-	-	1	1	1
8	*Brachyglottis repanda*	1	1	1	1	1	2	0	-	-	0	-	?
9	ditto	1	?	?	?	1	2	0	-	-	0	-	?
13	*Blennosperma nanum*	1	2	1	1	0	-	1	1	1	0	-	?
17	*Abrotanella emarginata*	0	-	-	2	1	1	0	-	-	0	-	0
19	*Liabum ovatum*	1	3	2	1	1	2	0	-	-	0	-	?
24	*Vernonia poskeana*	1	2	1	1	1	-	1	1	2	0	-	0
25	ditto	1	2	1	1	1	-	1	1	2	0	-	0
26	ditto	1	2	1	1	1	-	1	1	2	0	-	0
30	*V. pauciflora*	1	3	1	-	1	2	0	2	-	1	2	0